Article

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Exploring the lncRNA NEAT1/iASPP Pathway in Chordoma: Mechanisms of Proliferation Suppression and Apoptosis Induction

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Background: Chordoma is a rare, primary malignant bone tumor arising from remnants of embryonic notochord tissue. While the long non-coding RNA (lncRNA) nuclear paraspeckle assembly transcript 1 (*NEAT1*) has been implicated in various cancers, its role in chordoma remains to be elucidated. This study aims to elucidate the mechanisms by which lncRNA *NEAT1* influences chordoma growth and apoptosis.

Method: lncRNA *NEAT1* expression was assessed in chordoma using reverse transcription-quantitative polymerase chain reaction (RT-qPCR). Two chordoma cell lines with high lncRNA *NEAT1* expression were selected for lncRNA *NEAT1* knockdown and inhibitor of apoptosis-stimulating protein of p53 (iASPP) overexpression experiments. Cell survival was assessed using Cell Counting Kit-8 (CCK-8) and colony formation assays, while apoptosis and iASPP expression levels were analyzed by flow cytometry, Western blotting (WB), and RT-qPCR. *In vivo*, treated U-CH1 cell lines were subcutaneously injected into nude mice to establish a chordoma model. Tumor apoptosis was evaluated through hematoxylin-eosin (HE) staining and terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling (TUNEL) assay and WB was used to measure the levels of apoptosis-related proteins and iASPP in the tissues.

Results: The lncRNA *NEAT1* was highly expressed in chordoma. Silencing lncRNA *NEAT1* inhibited the survival and proliferation of chordoma cell lines MUG-Chor1 and U-CH1, and promoted apoptosis ($p < 0.05$). Overexpression of iASPP counteracted these effects, enhanced cell growth and inhibited apoptosis in the si-lncRNA NEAT1 group ($p < 0.05$). HE and TUNEL staining results indicated that silencing lncRNA *NEAT1* reduced cell proliferation and division in chordoma tissues, and led to a marked increase in apoptosis ($p < 0.05$). These effects were reversed by overexpression of iASPP ($p < 0.05$).

Conclusion: In chordoma, lncRNA *NEAT1* is highly expressed, and silencing lncRNA *NEAT1* inhibits the proliferation and induces apoptosis of chordoma cells while decreasing the expression of iASPP. These findings offer new insights into the involvement of the lncRNA *NEAT1*/iASPP pathway in chordoma development.

Keywords: lncRNA *NEAT1*; iASPP; programmed cell death; chordoma

Introduction

Chordoma, a rare and highly invasive bone sarcoma, exhibits infiltrative growth characteristics, particularly in the skull base and sacrum [1]. The most common early symptom is pain, and its involvement in joints may lead to instability while standing and pain during joint loading [2]. However, our understanding of chordoma's etiology and developmental mechanisms remains limited, contributing to the current constraints in diagnostic and therapeutic approaches. Therefore, a comprehensive exploration of the molecular mechanisms underlying chordoma and an active search for novel diagnostic, therapeutic, and preventive measures are of paramount clinical significance for improving treatment outcomes.

Long non-coding RNAs (lncRNAs) have recently garnered widespread attention as novel therapeutic targets [3]. Despite lacking protein-coding capacity, these RNAs play crucial roles in cell proliferation and apoptosis [4]. Aberrant expression of lncRNAs is intricately linked to the onset, progression, and metastasis of various tumors. Among them, nuclear paraspeckle assembly transcript 1 (*NEAT1*) is a lncRNA that has emerged as an oncogene of interest in multiple cancers, modulating key cancer-related processes, including cell growth and programmed cell death [5]. The dysregulation of NEAT1 has been confirmed in multiple malignancies such as glioma and liver cancer [6,7], making it a potential biological marker for tumor diagnosis and treatment.

In tumors, extensive research has been conducted on inhibitor of apoptosis-stimulating protein of p53 (iASPP)

[8]. The expression of iASPP is closely associated with late-stage malignant tumors, tumor recurrence, and poor prognosis. Chordoma, characterized by a poor prognosis and a high recurrence rate, has been the focus of previous studies that revealed a correlation between iASPP expression and clinical outcomes in chordoma, influencing cell proliferation and apoptosis [9]. iASPP typically interacts with p53, inhibiting p53-mediated programmed cell death (apoptosis) and impeding p53's regulation of downstream genes. This disruption results in a surge in anti-apoptotic protein B-cell lymphoma 2 (Bcl-2) expression and a decline in pro-apoptotic protein Bcl-2-associated X protein (Bax) expression. This imbalance makes chordoma cells more prone to evading apoptosis, promoting abnormal proliferation, and facilitating cancer cell survival [10,11]. p53, a key tumor suppressor, frequently mutates in cancer, disrupting its role in regulating cell cycle arrest and apoptosis [12]. NEAT1, regulated by p53, plays a crucial role in modulating apoptosis by influencing key cellular signaling pathways, which contribute to tumor progression and survival.

However, the mechanisms governing the reciprocal regulation between iASPP and lncRNA *NEAT1* in chordoma remain poorly understood. This study investigates the function of lncRNA *NEAT1* in chordoma, unveils its relationship with iASPP, and delves into the mechanisms by which it regulates the biological functions of chordoma. Through this research, we aim to identify new targets for treating chordoma and to gain a deeper understanding, which can establish a foundation for clinical trials.

Materials and Methods

Clinical Research

This study collected tumor tissue specimens from 31 patients diagnosed with chordoma. Specimens of adjacent normal tissues were obtained from regions at least 3 cm away from the tumor edge. All specimens were obtained from surgical resections, and patients who underwent chemotherapy or radiotherapy were excluded from this study. General data for patients are shown in Table 1.

Table 1. General data of patients with chordoma.

Factor	Patients (n = 31)
Age (years)	57.71 ± 4.98
Gender (male/female)	22/9
Pathological type	
Transitional epithelial carcinoma	20
Adenocarcinoma	7
Squamous carcinoma	4
Maximum tumor diameter	
≥3 cm	18
<3 cm	13
TNM stage	
<IIb stage	23
≥IIb stage	8

TNM stage, Tumor Node Metastasis Staging System.

Cell Culture

Nucleus pulposus cells (CP-H097, Pricella, Wuhan, China) and chordoma cell lines JHC7 (Johns Hopkins University, Baltimore, MD, USA), MUG-Chor1, and U-CH1 (YS1101C, YS1543C, YaJi Biological, Shanghai, China) were purchased. Cells were cultured in a mixture of Roswell Park Memorial Institute (RPMI) 1640 (R8758, Thermo Fisher Scientific, Waltham, MA, USA) and Dulbecco's Modified Eagle Medium (DMEM) (51435C, Thermo Fisher Scientific, Waltham, MA, USA) in a 1:4 ratio, supplemented with 1% L-glutamine, 10% fetal bovine serum (FBS) (C0235, Beyotime, Shanghai, China), and 1% penicillin/streptomycin (C0222, Beyotime, Shanghai, China). Cells were maintained in a constant temperature and humidity incubator at 37 °C with 5% CO_2. The medium was replaced every 2 or 3 days, ensuring cells reached 80% confluence before proceeding with the transfection. Before the experiment, all cells underwent short tandem repeat (STR) identification and mycoplasma detection to ensure that the STR identification of all cell lines was consistent with the reference values in the database and that no signs of mycoplasma infection were detected. All procedures followed aseptic techniques to prevent cell contamination.

Cell Transfection

The iASPP cDNA (GenePharma, Shanghai, China), was integrated into the human U6 promoter-multiple cloning site-ubiquitin-enhanced green fluorescent protein-internal ribosome entry site-puromycin (hU6-MCS-Ubiquitin-EGFP-IRES-puromycin) vector (GV428), creating the iASPP expression construct. The engineered plasmid was then co-transfected with lentiviral packaging plasmids (Genepharm, Shanghai, China) into HEK-293 cells to produce viral particles. Following filtration and concentration (10^8 TU/mL), the viral particles were introduced into chordoma cells. Infected cells were cultured for 5 or 6 additional passages (12–15 days) in a selection medium containing 5 µg/mL puromycin. Quantitative polymerase chain reaction (qPCR) was performed to verify iASPP overexpression, along with an overexpression negative control. GenePharma designed and synthesized small interfering RNA (si-RNA) targeting long intergenic non-coding RNA nuclear paraspeckle assembly transcript 1 (LINC NEAT1) as well as a small interfering RNA negative control (si-NC). The si-RNA solutions were mixed and added to cell culture plates or flasks containing culture medium, followed by gently shaking. Subsequent experiments were performed after 24 h of incubation under the specified conditions. The sequences of the si-RNAs are provided in Table 2.

Table 2. Primer sequences of si-LINC NEAT1.

si-RNA	Target Sequence (5′-3′)
si-LINC NEAT1	Sense: CAGGACUAGGUGCGUAGUGTT
	Antisense: CAGGACUAGGUGCGUAGUGTT
si-NC	Sense: AGCTTACGCTGAGCTAGCTA
	Antisense: TCGAATGCGACTCGATCGAT

LINC NEAT1, intergenic non-coding RNA nuclear paraspeckle assembly transcript 1; si-RNA, small interfering RNA; si-NC, small interfering RNA negative control.

CCK-8

After 48 h post-transfection, cells were collected and plated in a 96-well culture plate at a density of 5×10^3 cells per well. Next, 10 µL of Cell Counting Kit-8 (CCK-8) solution (40203ES60, Yesen Biotechnology, Shanghai, China) was added to each well. Cells were incubated at 37 °C with 5% CO_2 for 1 h and then the optical density was determined at 450 nm using a Multiskan FC microplate photometer (Thermo Fisher Scientific, Waltham, MA, USA).

Clone Formation Assay

Cells were plated in culture dishes at a density of 3×10^3 cells per well. After 2 weeks of incubation, cell colonies were fixed using 4% paraformaldehyde (PFA) (MM1504-100ML, Shanghai Maokang Biotechnology Co., LTD) and stained with 0.5% crystal violet. Cell colonies were washed and dried and then the number of cell colonies was manually counted.

Flow Cytometry

Transfected cells (2×10^4 cells/mL) were incubated at 37 °C with 5% CO_2 for 48 h. The cells were then washed twice with phosphate-buffered saline (PBS), centrifuged, and resuspended in binding buffer. They were incubated with Annexin V-FITC/PI in darkness for 15 minutes. Finally, Apoptotic cells were identified using a BD FACSCanto™ II flow cytometry (BD Biosciences, Franklin Lakes, NJ, USA), and data analysis was performed with CELLQuest 3.0 software (BD Biosciences, Franklin Lakes, NJ, USA).

Western Blotting

Protein extraction from cells was accomplished using Radioimmunoprecipitation Assay (RIPA) lysis buffer (P0013B, Beyotime, Shanghai, China). The protein concentration was assessed with the Bicinchoninic acid (BCA) protein analysis kit (P0012, Beyotime, Shanghai, China). Proteins were separated by 10% sodium dodecyl-sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to a polyvinylidene fluoride (PVDF) membrane. The PVDF membrane was blocked using 5% skim milk for 1 h. Incubation was carried out at 4 °C overnight with the following primary antibodies: Bcl-2 (1:1000, ab32124, all from Abcam, Cambridge, MA, USA), Bax (1:1000, ab32503), p53 (1:1000, ab32509), iASPP (1:1000, ab34898), and Glyceraldehyde-3-Phosphate Dehydrogenase (GAPDH) (1:1000, ab8245). After washing with Tris Buffered Saline with Tween 20 (TBST), the membrane was incubated with goat anti-rabbit immunoglobulin G (IgG) heavy and light chain (H&L) horseradish peroxidase (HRP) conjugate (1:2000, ab205718, Abcam) and goat anti-mouse IgG H&L (HRP) (1:2000, ab205719, Abcam)for 1 h, followed by additional TBST washes. Enhanced chemiluminescence (ECL) reagent (KGP1128, Shanghai ZenBio Science and Technology Co., Ltd., Shanghai, China) was used to detect the protein bands. Protein expression levels were analyzed using ImageJ software (version 8.0; National Institutes of Health, Bethesda, MD, USA).

RT-qPCR

Tissues and cells were subjected to RNA extraction using Trizol reagent (15596018CN, Invitrogen, Waltham, CA, USA). Next, the PrimeScript RT reagent kit (RR014A, Takara, Osaka, Japan) was used for reverse transcription, while the SYBR Prime Script reverse transcription-quantitative polymerase chain reaction (RT-qPCR) kit (Takara, Osaka, Japan) was used for RT-qPCR, according to the manufacturer's instructions. Results were quantified employing the $2^{-\Delta\Delta Ct}$ approach. Each experiment was conducted in triplicate, and the fold change in expression levels relative to the respective controls was defined as 1.0. The primer sequences are shown in Table 3.

Animal Experiments

In brief, 25 male BALB/c nude mice (Slack, Jingda, Changsha, China), aged 4–6 weeks and weighing 18–20 g, were purchased. Mice were maintained under specific pathogen-free conditions. Subsequently, 1×10^6 U-CH1 cells were transfected with LINC NEAT1 and iASPP subcutaneously near the left thigh of mice. Mice were divided into Control, si-NC, si-LncRNA NEAT1, si-LncRNA NEAT1+oe-NC, and si-LncRNA NEAT1+oe-iASPP. There were 5 mice per group. After 3 weeks, mice were euthanized by cervical dislocation under 2–3% isoflurane anesthesia, and tumor tissues were collected.

Hematoxylin-eosin (HE) Staining

The tissues were fixed in a 10% formalin solution, followed by dehydration in an ethanol gradient. Next, samples were embedded in paraffin and prepared into 4 µm-thick sections. After sectioning, staining was performed using hematoxylin-eosin (HE) (R03040, Sigma Aldrich, St. Louis, MO, USA). Sections were examined using an optical microscope (DM1000, Leica Microsystems, Wetzlar, Germany).

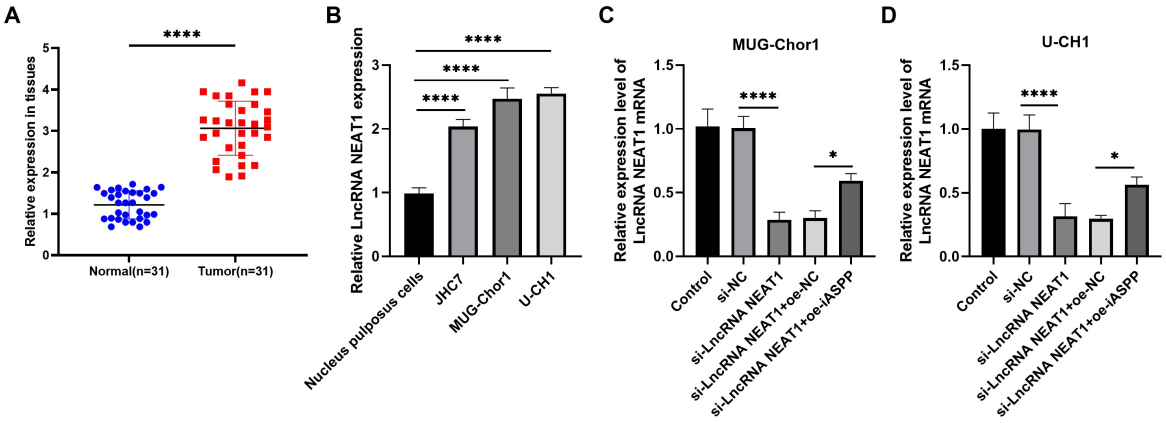

Fig. 1. Overexpression of iASPP promotes long non-coding RNA (lncRNA) *NEAT1* expression in chordoma. (A) reverse transcription-quantitative polymerase chain reaction (RT-qPCR) detection of lncRNA *NEAT1* expression in chordoma tissues and adjacent tissues. (B) RT-qPCR detection of lncRNA *NEAT1* expression in chordoma cell lines. (C,D) RT-qPCR detection of lncRNA *NEAT1* expression in MUG-Chor1 and U-CH1 cell lines. n = 3. *$p < 0.05$, ****$p < 0.0001$.

Table 3. Primer sequences of LINC NEAT1 and iASPP.

	Forward primer	Reverse primer
LINC NEAT1	AGTTTGAAAAGGCTAATCCAG	GACAACAGGCTAACTAACCC
iASPP	GGCGGTGAAGGAGATGAAC	TGATGAGGAAATCCACGATAGAG
GAPDH	GACAGTCAGCCGCATCTTCT	GCGCCCAATACGACCAAATC

iASPP, inhibitor of apoptosis-stimulating protein of p53; GAPDH, Glyceraldehyde-3-Phosphate Dehydrogenase.

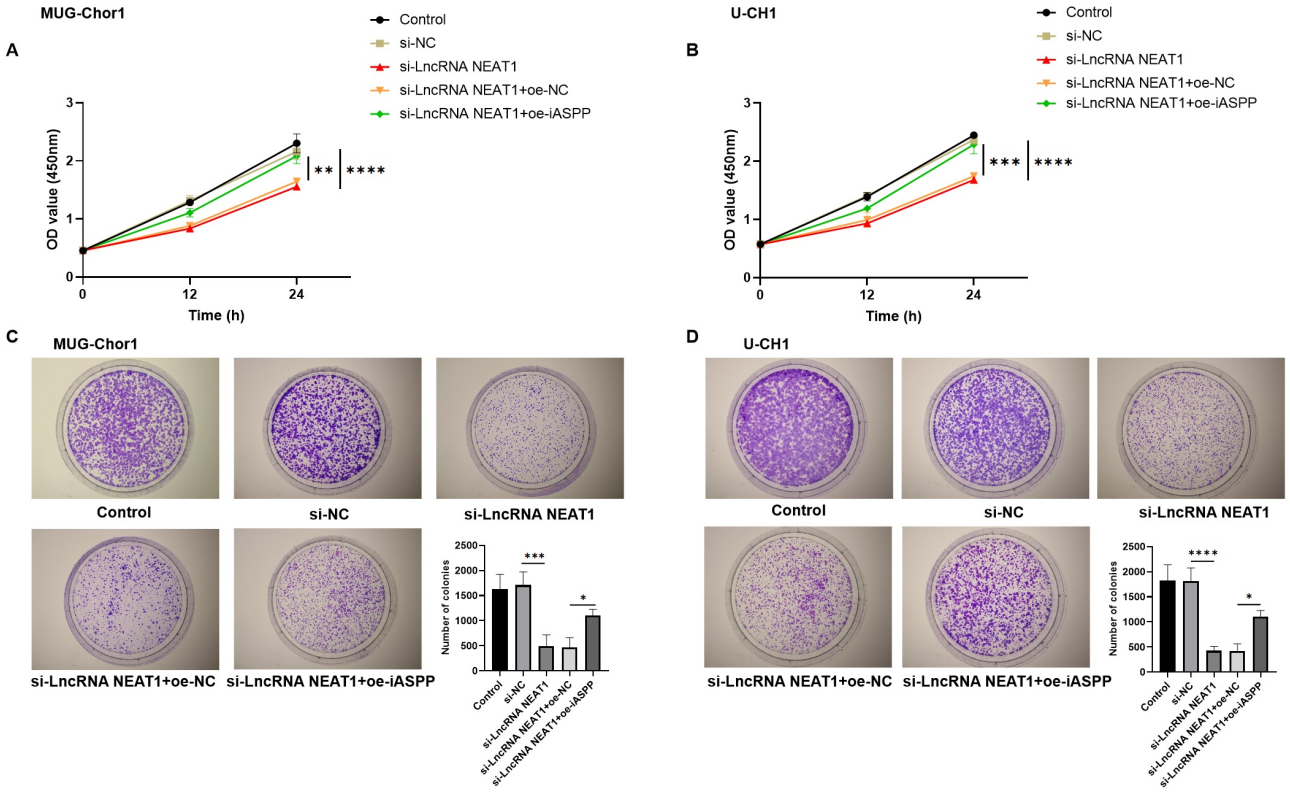

Fig. 2. iASPP overexpression reverses lncRNA *NEAT1* silencing-induced growth inhibition in chordoma cells. (A,B) Cell Counting Kit-8 (CCK-8) was used to detect cell viability. (C,D) A clone formation assay was used to detect cell proliferation. n = 3. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$.

TUNEL Staining

The dewaxed tissue samples underwent incubation with 0.1% Triton X-100 (P0096, Beyotime, Shanghai, China), followed by rinsing in PBS. Next, samples were subjected to staining using the terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling (TUNEL) apoptosis detection kit (40306ES20, Yesen Biotechnology, Shanghai, China), according to the manufacturer's instructions. TUNEL-positive signals (red spots) were counted as apoptotic cells using a fluorescence microscope (BX63, IX81, Olympus, Tokyo, Japan).

Statistical Analysis

Data are expressed as mean ± standard deviation. Statistics were performed utilizing GraphPad Prism software version 8.0 (GraphPad Software Inc., San Diego, CA, USA). Comparisons between two groups were conducted using Student's *t*-test, while comparisons among multiple groups were performed using one-way analysis of variance (ANOVA) followed by Tukey's post hoc test. Statistical significance was considered achieved for *p*-values < 0.05.

Results

Overexpression of iASPP Promotes lncRNA NEAT1 Expression in Chordoma

RT-qPCR analysis was conducted on tumor tissue and adjacent tissue samples from 31 patients with chordoma. It was observed that lncRNA *NEAT1* expression was significantly upregulated in chordoma tissues compared to adjacent tissues (Fig. 1A, $p < 0.05$). Furthermore, when compared to nucleus pulposus cells, the expression levels of lncRNA *NEAT1* in the chordoma cell lines JHC7, MUG-Chor1, and U-CH1 were significantly elevated (Fig. 1B, $p < 0.05$). Subsequently, two chordoma cell lines, MUG-Chor1 and U-CH1, with higher lncRNA *NEAT1* expression were selected for further experiments. These cell lines were transfected with si-lncRNA NEAT1 and oe-iASPP, and the expression of lncRNA *NEAT1* in the cells was assessed using RT-qPCR. The results showed that there was no significant difference in lncRNA *NEAT1* expression between the si-NC group and the control group ($p > 0.05$). However, the si-lncRNA NEAT1 group had significantly lower lncRNA *NEAT1* expression compared to the si-NC group. Furthermore, when compared to the si-lncRNA NEAT1+oe-NC group, the si-lncRNA NEAT1+oe-iASPP group exhibited increased lncRNA *NEAT1* expression (Fig. 1C,D, $p < 0.05$).

iASPP Overexpression Reverses lncRNA NEAT1 Silencing-Induced Growth Inhibition in Chordoma Cells

The results showed that in the chordoma cell lines MUG-Chor1 and U-CH1, there was no significant difference in cell viability and proliferation between the si-NC group and the control group ($p > 0.05$). However, when compared to the si-NC group, both cell viability and proliferation were significantly reduced in the si-LncRNA NEAT1 group ($p < 0.05$). Additionally, when compared to the si-lncRNA NEAT1+oe-NC group, the si-lncRNA NEAT1+oe-iASPP group demonstrated enhanced cell viability and proliferation (Fig. 2A–D, $p < 0.05$).

Overexpression of iASPP Reversed the Pro-apoptotic Effect of lncRNA NEAT1 Silencing on Chordoma Cells

In the MUG-Chor1 and U-CH1 cell lines, the si-NC group showed no significant changes in the apoptosis rate or the expression levels of Bax, p53, and Bcl-2 compared to the control group ($p > 0.05$). However, when compared to the si-NC group, the si-lncRNA NEAT1 group exhibited higher apoptosis rates, increased expression of Bax and p53, and decreased expression of Bcl-2 ($p < 0.05$). Additionally, when compared to the si-lncRNA NEAT1+oe-NC group, the si-lncRNA NEAT1+oe-iASPP group demonstrated increased Bcl-2 expression, reduced apoptosis rates, and lower Bax and p53 expression (Fig. 3A–D, $p < 0.05$).

Silencing lncRNA NEAT1 Inhibited iASPP Expression in Chordoma Cells

In MUG-Chor1 and U-CH1 cells, there was no significant change in iASPP expression in the si-NC group compared with the control group ($p > 0.05$). Compared to the si-NC group, the expression of iASPP was suppressed in the si-lncRNA NEAT1 group. Additionally, compared to the si-lncRNA NEAT1+oe-NC group, iASPP expression was increased in the si-lncRNA NEAT1+oe-iASPP group (Fig. 4A–D, $p < 0.05$).

Overexpression of iASPP Reversed the Pro-apoptotic Effect of si-lncRNA NEAT1 on Chordoma Tissue

Compared to the model group, the staining results of tumor tissues in the Model+si-NC group showed no significant changes ($p > 0.05$). In contrast to the Model+si-NC group, the Model+si-lncRNA NEAT1 group exhibited more loosely arranged tumor cells, reduced mitotic activity, lighter nuclear chromatin, and an increased number of apoptotic cells ($p < 0.05$). Compared to the si-lncRNA NEAT1+oe-NC group, the Model+si-lncRNA NEAT1+oe-iASPP group had more densely packed tumor cells, darker nuclear chromatin, and fewer apoptotic cells (Fig. 5A,B, $p < 0.05$).

Overexpression of iASPP Reversed the Pro-apoptotic Effects of si-lncRNA NEAT1 and its Suppression of iASPP Expression

The results showed that, compared to the Model group, the Model+si-NC group had no significant changes in the expression of apoptotic proteins and iASPP ($p > 0.05$). Compared to the Model+si-NC group, the Model+si-

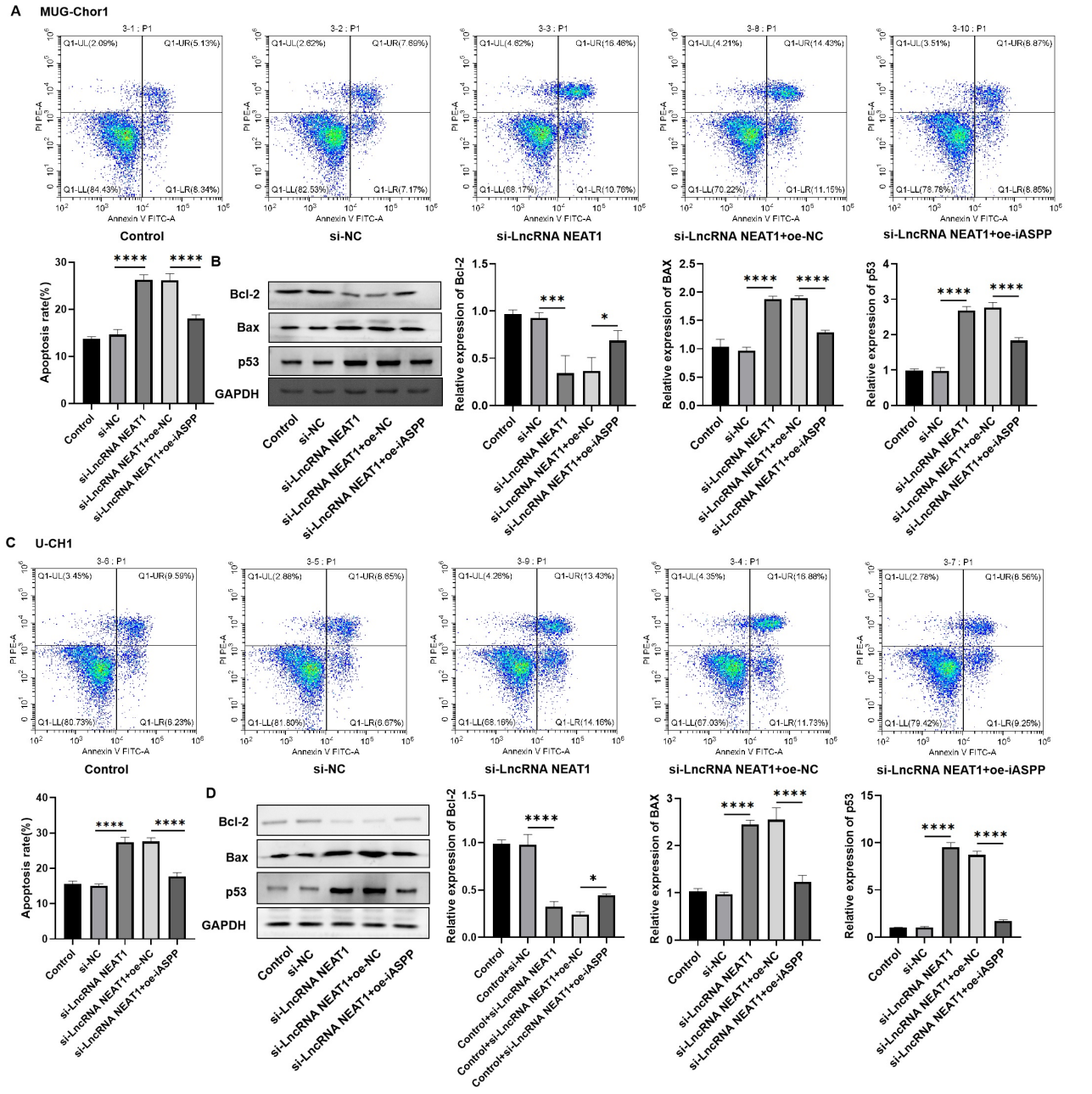

Fig. 3. Overexpression of iASPP reversed the pro-apoptotic effects induced by lncRNA *NEAT1* silencing in chordoma cells. (A) The apoptosis rate of MUG-Chor1 cells was detected by flow cytometry. (B) Western blotting analyzed the protein levels of B-cell lymphoma 2 (Bcl-2), Bcl-2-associated X protein (Bax), and p53 in MUG-Chor1 cells. (C) The apoptosis rate of U-CH1 cells was detected by flow cytometry. (D) Protein levels of Bcl-2, Bax, and p53 in U-CH1 cells were analyzed by Western blotting. n = 3. *$p < 0.05$, ***$p < 0.001$, ****$p < 0.0001$.

lncRNA NEAT1 group had reduced expression of Bcl-2 and iASPP, while the levels of Bax and p53 were increased ($p < 0.05$). Compared to the Model+si-lncRNA NEAT1+si-NC group, the Model+si-lncRNA NEAT1+oe-iASPP group exhibited increased expression of Bcl-2 and iASPP, and decreased expression of Bax and p53 (Fig. 6A,B, $p < 0.05$).

Discussion

Chordoma typically arises in tissues near the spinal cord or spinal nerve roots [12,13]. While the incidence rate is relatively low, chordoma can occur at any age, with certain types being more prevalent in specific age groups [14–16]. The treatment of spinal cord tumors encompasses various aspects, with molecular therapy gaining interest in

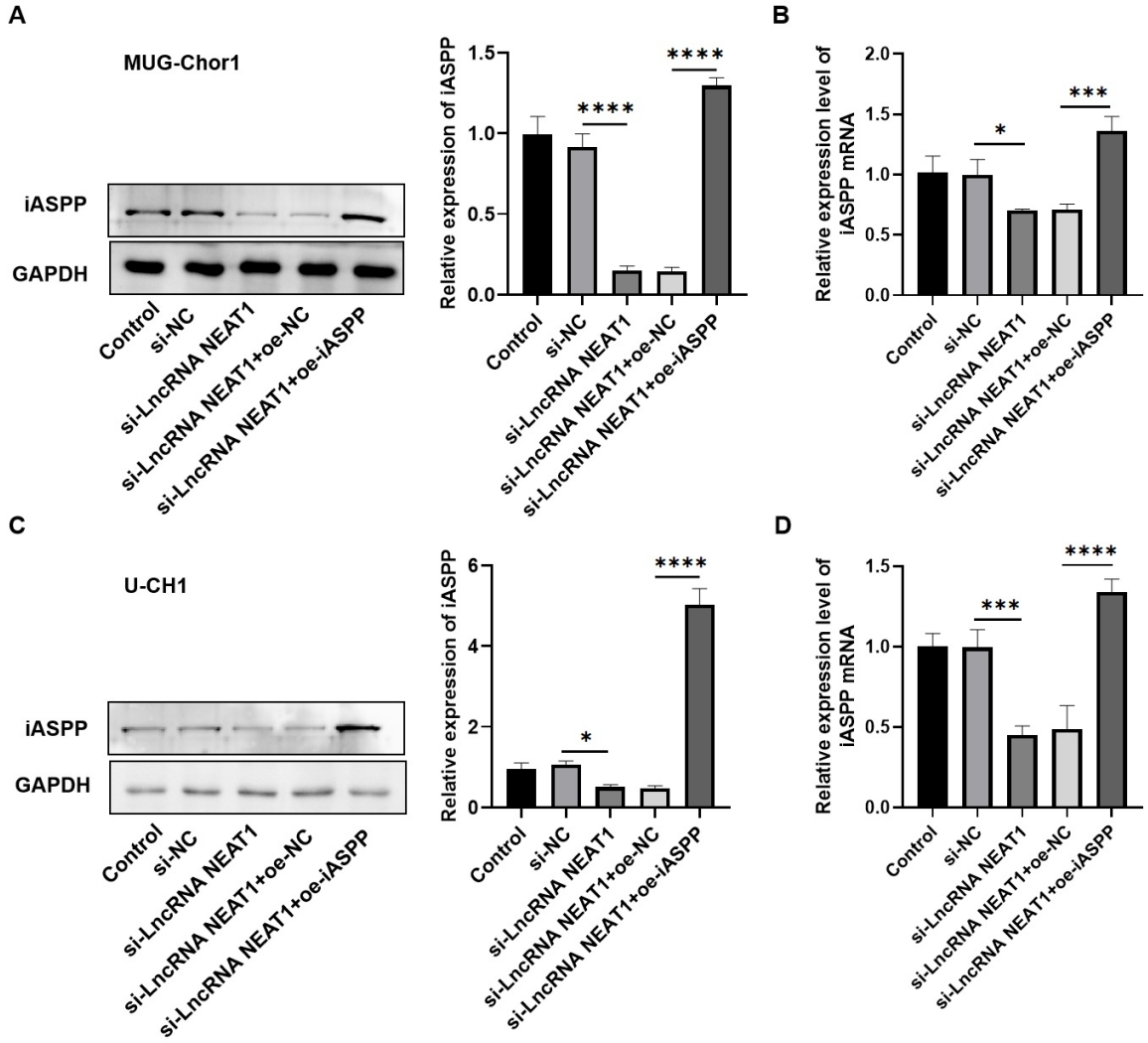

Fig. 4. Silencing lncRNA *NEAT1* inhibited iASPP in chordoma cells. (A,B) Western blotting and RT-qPCR were employed to examine iASPP expression in MUG-Chor1 cells. (C,D) iASPP expression in U-CH1 cells was assessed using Western blotting and RT-qPCR. n = 3. *$p < 0.05$, ***$p < 0.001$, ****$p < 0.0001$.

recent years [17,18]. In-depth exploration of the molecular mechanisms underlying chordoma, along with novel diagnostic, therapeutic, and preventive strategies hold significant clinical importance for advancing the standard of care.

Studies have shown that the expression level of NEAT1 is abnormally elevated in liver cancer [19,20]. Increased NEAT1 levels are associated with apoptosis. Overexpression of NEAT1 can inhibit the expression of apoptosis-related genes and proteins, slowing the apoptotic process and thereby promoting cancer cell survival [21–23]. The expression of NEAT1 in cancer is associated with the proliferation and apoptosis of cancer cells, and further studies are needed to elucidate its mechanism of action. These studies may provide new insights into how to diagnose cancer earlier and treat the disease more effectively. Research indicates that in certain types of spinal cord tumors, the expression levels of iASPP are significantly increased [24]. The overexpression of iASPP correlates with the promotion of cell proliferation and the inhibition of apoptosis. By interacting with p53, iASPP suppresses the transcriptional activity of p53, thereby reducing p53-mediated cell apoptosis [25]. This may promote faster tumor growth and render tumor cells resistant to treatment. The expression of iASPP in spinal cord tumors and its role in tumor biology make it a potential target for therapeutic intervention.

This study revealed that lncRNA *NEAT1* is significantly upregulated in bladder cancer tissues, and overexpression of iASPP can reverse the downregulation of lncRNA *NEAT1*. This finding suggests that iASPP may influence NEAT1 expression by regulating upstream signaling pathways or participating in feedback loops. As a known anti-apoptotic factor, iASPP may indirectly modulate NEAT1 transcriptional activity or stability through interactions with key transcription factors or signaling molecules. Research shows that silencing NEAT1 or overexpressing miR-124 suppresses iASPP expression [26,27].

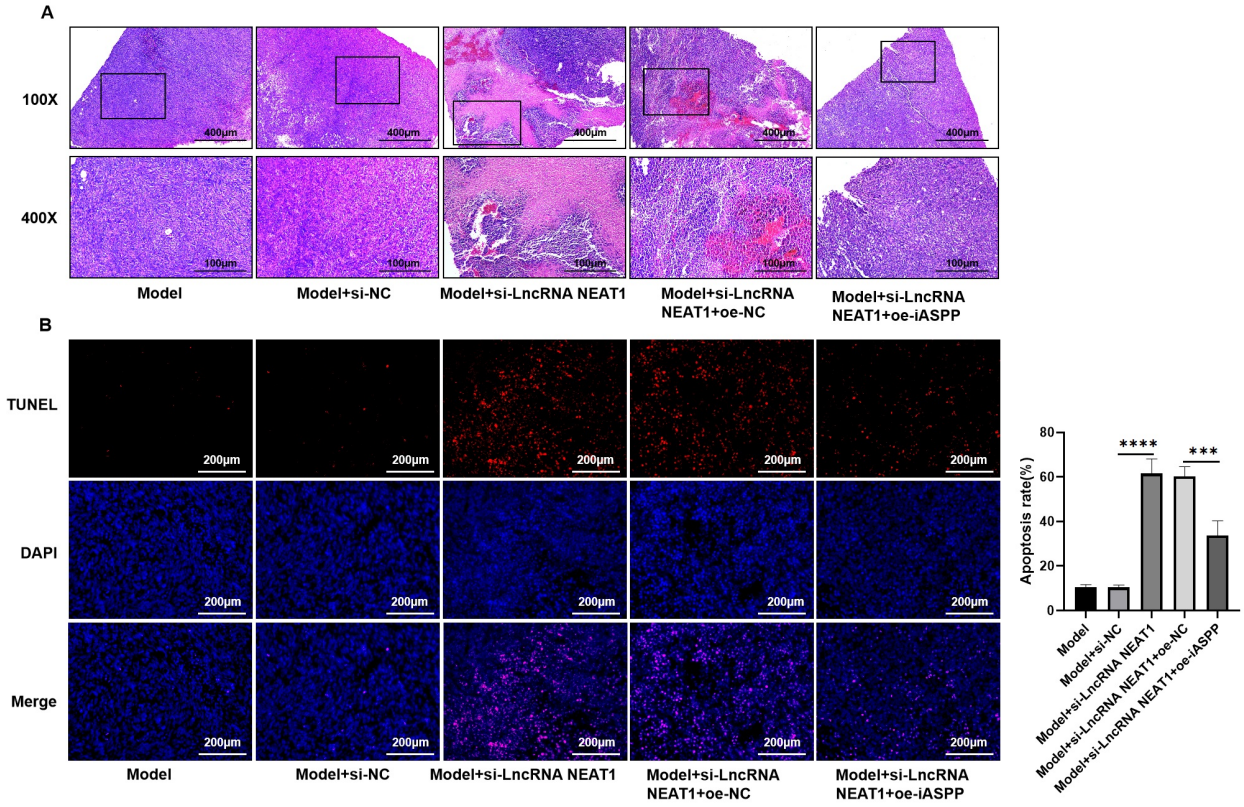

Fig. 5. Overexpression of iASPP reversed the pro-apoptotic effect of si-lncRNA NEAT1 on chordoma tissue. (A) Hematoxylin-eosin (HE) staining was used to evaluate the histopathology of chordoma. (B) Apoptosis of chordoma cells was detected by terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling (TUNEL). n = 5. ***$p < 0.001$, ****$p < 0.0001$.

This indicates that iASPP could be involved in the regulation of NEAT1 through complex feedback mechanisms, thereby regulating certain cellular processes.

To explore the regulatory function of lncRNA *NEAT1* in cell proliferation and apoptosis in chordoma, we employed NEAT1 silencing and iASPP overexpression to observe their impact on the biological behavior of chordoma cells. Experimental findings from NEAT1 silencing experiments using si-RNA showed significant inhibition in the growth and proliferation of cancer cells in the si-lncRNA NEAT1 group compared to the control group, concurrently promoting apoptosis and resulting in elevated levels of apoptotic proteins (Bax and p53). The expression of iASPP in cells of the si-lncRNA NEAT1 group significantly decreased. Subsequently, we further investigated the influence of silencing lncRNA *NEAT1* followed by overexpressing iASPP on cell behavior. The results revealed that, compared to the si-lncRNA NEAT1+oe-NC group, there was a significant increase in cell viability and proliferation in the si-lncRNA NEAT1+oe-iASPP group. Simultaneously, apoptosis was inhibited, and the expression of apoptotic proteins Bax and p53 in cells and tissues decreased, while that of iASPP increased. To validate whether this phenomenon holds true *in vivo*, we injected U-CH1 cells transfected with lncRNA *NEAT1* into mice and assessed their influence on apoptosis and iASPP expression within tumor tissues. The study results indicated a notable increase in tumor apoptosis in the si-lncRNA NEAT1 group compared to the model group, accompanied by an upregulation in Bax and p53 expression and a downregulation of iASPP expression. In summary, our research findings reveal that in chordoma, elevated levels of lncRNA *NEAT1* foster cell proliferation, impede apoptosis, and intensify iASPP expression. This discovery offers crucial experimental evidence to further comprehend chordoma's pathogenesis and explore innovative therapeutic strategies.

These findings offer crucial clues to enhance our understanding of the molecular mechanisms of chordoma. NEAT1, by regulating iASPP, impacts cell survival and proliferation, offering potential new targets for chordoma therapy. Further research will contribute to revealing the specific roles of the NEAT1/iASPP pathway in the initiation and progression of chordoma, providing a more profound insight into the development of therapeutic strategies. Nevertheless, it's crucial to highlight that, notwithstanding our study presenting compelling support for the pivotal role of NEAT1 in chordoma, further validation is required using additional clinical samples and cell lines, along with a more in-depth exploration of the detailed mechanisms of the NEAT1/iASPP pathway.

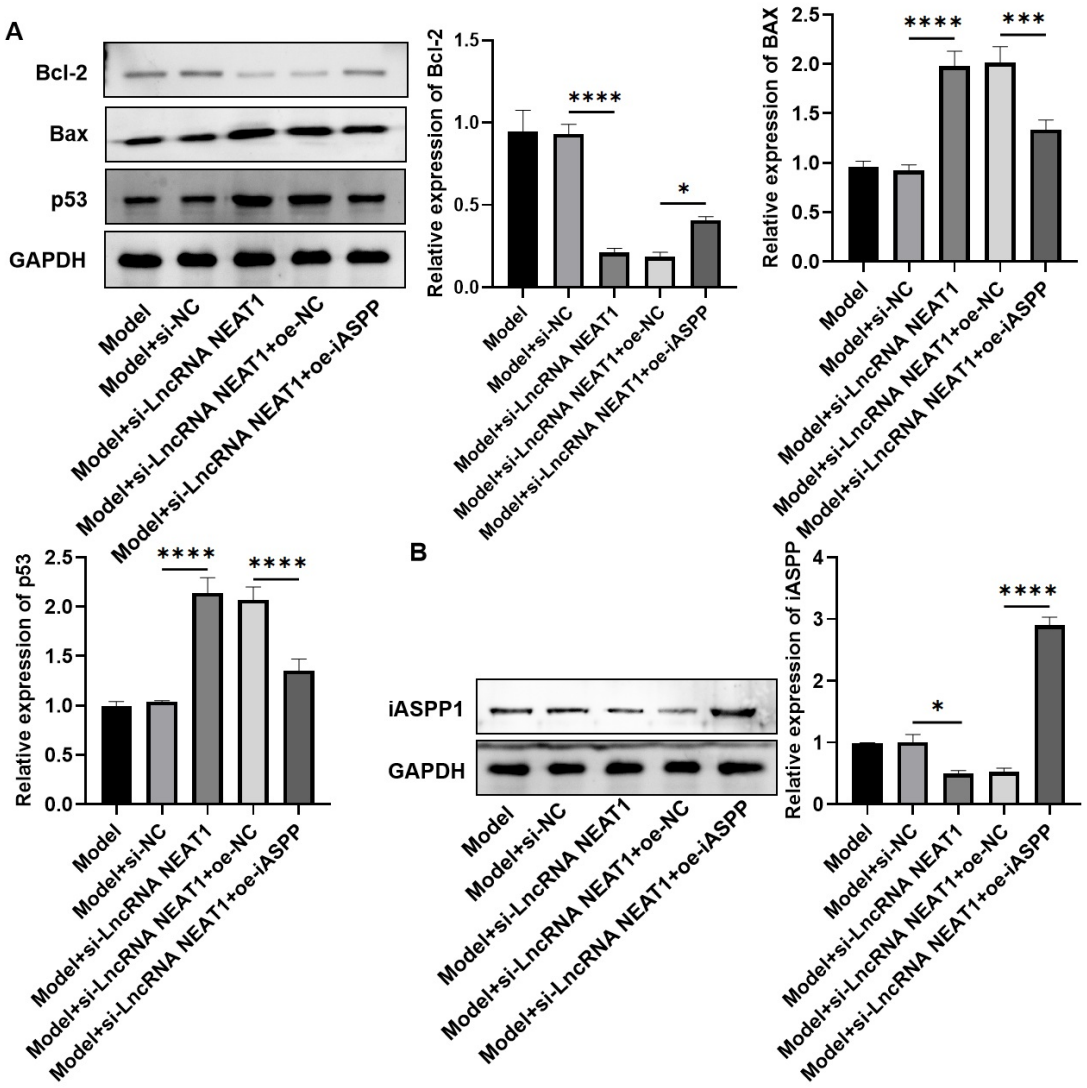

Fig. 6. Overexpression of iASPP reversed the pro-apoptotic effects of si-lncRNA NEAT1 and its suppression of iASPP expression. (A) The protein levels of Bcl-2, Bax, and p53 were detected by Western blotting. (B) The expression of iASPP in chordoma was detected by Western blotting. n = 5. *$p < 0.05$, ***$p < 0.001$, ****$p < 0.0001$.

Conclusion

In chordoma, lncRNA *NEAT1* is highly expressed, and silencing lncRNA *NEAT1* inhibits the proliferation and apoptosis of chordoma cells while decreasing the expression of iASPP. These findings offer fresh perspectives on the involvement of the lncRNA *NEAT1*/iASPP pathway in chordoma development.

Availability of Data and Materials

The data used and analyzed during the current study are available from the corresponding authors.

Author Contributions

ZGM, LW and ZJ designed the research study and wrote the original manuscript. LW and ZJ performed the research. ZGM and HWL provided help and advice on the experiments. HWL analyzed the data. All authors were involved in the drafting and critical revision of the manuscript. All authors read and approved the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

All patients provided written consent after being informed. This study was approved by the ethics committee of People's Hospital of Xinjiang Uygur Autonomous Region (No. KY2023062008) and followed the principles of the Declaration of Helsinki. All experimental protocols involving animals were approved by the Institutional Animal Care and Use Committee of the Animal Ethics Committee

of People's Hospital of Xinjiang Uygur Autonomous Region (No.XJ20230215) to ensure compliance with ethical guidelines for laboratory animal care and use.

Acknowledgment

Not applicable.

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Conflict of Interest

The authors declare no conflict of interest.

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